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Development of the Lateral and Medial Limbic Cortices in the Rat in Relation to Cortical Phylogeny

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[1H]Thymidine autoradiography was used to investigate neurogenesis of the lateral limbic cortex and morphogenesis of the medial and lateral limbic cortices in adult and embryonic rat brains. Ontogenetic patterns in the limbic cortex are unique because some neurogenic gradients are linked to those in neocortex, others are linked to those in paleocortex. These findings are related to hypotheses of cortical phylogeny. The experimental animals used for neurogenesis were the offspring of pregnant females injected with [1H]thymidine on 2 consecutive days: Embryonic Day (E)13–E14, E14–E15, ... E21–E22, respectively. On Postnatal Day (P)60, the proportion of neurons originating during 24-h periods were quantified at nine anteroposterior levels and one sagittal level. Similar to neocortex, deep cells are generated earlier than superficial cells throughout the lateral limbic cortex: layer VI mainly on E14–E15, layer V on E15–E16, and layers IV–II on E16–E18. There is a ventral/older to dorsal/younger neurogenic gradient between the ventral agranular insular, dorsal agranular insular, and gustatory cortical areas and between ventral and dorsal orbital areas beneath the frontal pole. Similar to paleocortex below the rhinal sulcus, limbic cortex in the rhinal sulcus has a “sandwich” gradient: the older posterior agranular insular area is sandwiched by anterior and posterior younger areas (ventral agranular insular and perirhinal). To study morphogenesis, pregnant females were given single injections of [1H]thymidine during gestation and embryos were removed in successive 24-h intervals (sequential-survival). Neurogenesis finishes first in ventral limbic areas, later in dorsal limbic areas, and latest in neocortical areas. The cortical plate in the region of the medial and lateral limbic cortices does not have a separate subplate layer as is found in the region of the neocortex. Instead, layer VI in the limbic cortices has unusually older cells that are generated simultaneously with subplate cells.

INTRODUCTION

As early as 1664, Thomas Willis wrote that the cortex on the borders of the cerebral hemispheres had unique anatomical features resembling a “hem” or “limbus.” In his 1878 paper, Broca called that area the “grande lobe limbique” (as reviewed in Ref. (45)). In rats and in man, the limbic lobe is a continuous cortical band encircling the neocortex. Phylogenetic relationships within the limbic lobe and with the neocortex were often the subjects of contradicting opinions and confusing terminology in the classical neuroanatomical literature (1, 2, 22, 40). None of the hypotheses has found general acceptance, and interest in the topic of evolutionary origin has waned in recent years. Since ontogenetic patterns are important clues to phylogenetic links, a comprehensive developmental study of the limbic lobe as a whole would shed new light on old controversies. In 1974, Bayer and Altman (10) started using the comprehensive labeling method of [1H]thymidine autoradiography to quantitatively determine timetables of neuronal birthdays throughout the rat nervous system. With data presented in a companion paper (9), our forthcoming book on neocortical development (13), previous work on the hippocampal region (6, 10) and primary olfactory cortex (8), quantitative developmental analyses of the entire telencephalic cortical mantle are complete. These studies show that each major cortical “system” has characteristic neurogenic timetables and gradients.

The developmental patterns in the limbic cortex are unique in that they are linked on the one hand to the neocortex and on the other to the paleocortex (piriform and entorhinal areas) and archicortex (hippocampus). Consequently, the first goal of this paper is to discuss overall ontogenetic patterns in the entire cortex in relation to hypotheses of phylogenetic origins. The second goal is to present quantitative timetables of neurogenesis in the insular, perirhinal, and gustatory cortices. Although some of these areas have been quantitatively studied (8), a complete genetic timetable has not been done. The third goal is to examine the embryonic development of the cortex, focusing on differences between center and edges. The unique location of unusually older deep cells in the medial and lateral extremes of the cortical plate may be linked to the distribution of dopamine axon terminals in the cortex.

MATERIALS AND METHODS

Long Survival [1H]Thymidine Autoradiography

The experimental animals were the offspring of Purdue–Wistar timed-pregnant rats given two subcuta-
neous injections of \[^{3}H\]thymidine (Schwarz-Mann; sp act, 6.0 Ci/mM; 5 \(\mu\)Ci/g body wt) to ensure that cells originating after the onset of the injections will be detected as labeled (comprehensive labeling). The injections (given between 8:30 and 9:00 AM) to an individual animal were separated by 24 h. Two or more pregnant females made up each injection group. The onset of the \[^{3}H\]thymidine injections was progressively delayed by 1 day between groups (E13–E14, E14–E15, . . . E21–E22) so that the amount of neurogenesis could be determined within a single 24-h period. The day the females were sperm positive was designated Embryonic Day 1 (E1). Normally, births occur on E23, which is also designated as Postnatal Day 0 (P0). All animals were perfused through the heart with 10% neutral (pH 7.4) formalin on P60. The brains were kept for 24 h in Bouin’s fixative and then were transferred to 10% neutral formalin until they were embedded in paraffin. The brains of at least six animals from each injection group were blocked coronally according to the stereotaxic angle of Pellegrino et al.’s (33) atlas. Every 15th section (6 \(\mu\)m) through the cerebral cortex was saved. The brains of some animals in each injection group were also sectioned in the sagittal plane (every 15th was saved) to examine the cortex in aZ.’s (33) atlas. Every 15th section through the cortical area was labeled (comprehensive labeling). The injections for the layer V cells in the perirhinal cortex at levels A4.2–A1.2 (bottom graph, Fig. 3). To get the height of the bar on E15, for example, the proportion of labeled cells in injection group E16–E17 (entry D, column 3) is subtracted from the proportion labeled cells in injection group E15–E16 (entry C, column 3) to get the proportion of cells originating during the day on E15 (63.31%).

Coronal sections were selected for quantitative analysis at nine anteroposterior levels (A9.2 to A1.2; drawings, Fig. 3), and sagittal sections at 1.5 mm lateral to the midline. Cells were counted microscopically at \(\times 312.5\) in unit areas set off by a \(10 \times 10\) ocular grid (0.085 \(\text{mm}^2\) per section). For quantification, all neurons within a designated area were assigned to one of two groups, labeled or nonlabeled. Cells with silver grains overlying the nucleus in densities above background levels were considered labeled. In our material, background noise is very low (approximately 2–4 grains per grid square), and a cluster of 6–12 silver grains over a nucleus (approximately 3X background) was enough to identify a labeled cell. However, due to the comprehensive labeling and the long exposure period, only a slight proportion of the neurons was lightly labeled. Obvious endothelial cells (crescent-shaped cells with pale nuclei surrounding capillaries) and glial cells (small cells with densely staining nuclei and indefinite nucleoli) were excluded from the counts. The proportion of labeled cells (percentage labeled cells/total cells) was then calculated from these data.

The determination of the proportion of cells arising (ceasing to divide) on a particular day used a modification of the progressively delayed comprehensive labeling procedure (10) and is described in detail elsewhere (11). Briefly, a progressive reduction in the proportion of labeled neurons from a maximal level (>95%) in a specific population indicates that the precursor cells are producing nonmitotic neurons. By analyzing the rate of decline in labeled neurons, one can determine the proportion of neurons originating over blocks of days (or single days) during development. Table 1 shows the data and calculations for the layer V cells in the perirhinal cortex at levels A4.2–A1.2 (bottom graph, Fig. 3).

Throughout the quantitative analysis, it was noted that even slight trends in cell labeling within animals were very consistent. In the dorsal agranular insular cortex, for example, the layer VI cells at levels A5.2–A7.2 tended to have a lower percentage of labeled cells than those at level A9.2 during the peak period of neurogenesis. That indicates that at least some posterior cells have birthdays earlier than those of anterior cells. Parametric statistical tests, such as a nonnested analysis of variance, look only at the degree of divergence between groups, and slight consistent differences within animals are disregarded. Consequently, the nonparametric sign test (17) was used to analyze cell labeling patterns. The sign test determines the consistency of sequential neuron production between paired locations within individual animals. The comparisons are grouped into three categories: (1) \(X > Y\), “+” comparison; (2) \(X < Y\), “−” comparison; and (3) \(X = Y\), “0” comparison. The zero comparisons are discarded and, depending on the total number of remaining “+” and “−” comparisons, either a binomial distribution or a normal approximation is used to calculate probabilities (P). The graphs throughout this report show the more variable group data rather than consistent trends in data from individual animals. Consequently, some of the statistically significant neurogenic gradients (between ventral and dorsal super-

**TABLE 1**

<table>
<thead>
<tr>
<th>Injection group</th>
<th>(N)</th>
<th>% Labeled cells (mean ± SD)</th>
<th>Day of origin</th>
<th>% Cells originating</th>
</tr>
</thead>
<tbody>
<tr>
<td>E13–E14</td>
<td>8</td>
<td>(A) 100 ± 0</td>
<td>E13</td>
<td>0.49 (A–B)</td>
</tr>
<tr>
<td>E14–E15</td>
<td>7</td>
<td>(B) 99.51 ± 0.33</td>
<td>E14</td>
<td>4.75 (B–C)</td>
</tr>
<tr>
<td>E15–E16</td>
<td>6</td>
<td>(C) 94.76 ± 1.29</td>
<td>E15</td>
<td>63.01 (C–D)</td>
</tr>
<tr>
<td>E16–E17</td>
<td>12</td>
<td>(D) 31.44 ± 7.65</td>
<td>E16</td>
<td>23.72 (D–E)</td>
</tr>
<tr>
<td>E17–E18</td>
<td>9</td>
<td>(E) 7.72 ± 2.41</td>
<td>E17</td>
<td>4.31 (E–F)</td>
</tr>
<tr>
<td>E18–E19</td>
<td>6</td>
<td>(F) 3.41 ± 1.01</td>
<td>E18</td>
<td>3.41 (F–G)</td>
</tr>
<tr>
<td>E19–E20</td>
<td>6</td>
<td>(G) 0 ± 0</td>
<td>E19</td>
<td>0</td>
</tr>
</tbody>
</table>

* The data for the deep cells in the perirhinal cortex are given as an example of how they are derived for presentation in the bar graphs used throughout the paper. \(N\) refers to the number of animals analyzed in each injection group. The percentage labeled cells for each injection group gives the group means ± the standard deviation for the raw data counts (percentage of labeled cells to total cells in individual animals). The standard deviations are typical of the variability seen throughout data collection. The percentage cells originating column lists the data that are presented in the bar graph (Fig. 3, bottom). To get the height of the bar on E15, for example, the proportion of labeled cells in injection group E16–E17 (entry D, column 3) is subtracted from the proportion labeled cells in injection group E15–E16 (entry C, column 3) to get the proportion of cells originating during the day on E15 (63.31%).
ficial cells, for example) are not conspicuous in the group data.

Short and Sequential Survival

\[^{3}H\]Thymidine Autoradiography

The experimental animals were the embryos from Purdue-Wistar timed-pregnant rats given a single subcutaneous injection of \[^{3}H\]thymidine (Schwarz-Mann; sp act, 6.0 Ci/mM; 5 μCi/g body wt) between 9 and 11 AM. The day of sperm positivity is designated as Gestation Day 1 (E1). Several dams were injected for each day between E13 and E21. Survival times in each injection group varied from 2 h (short survival series) to several days (sequential survival series). For example, one dam in the E15 injection group was killed 2 h after the injection, another was killed 1 day later on E16, another 2 days later on E17, and so on until the last dam was killed on E22. All groups were treated as the E15 group. The dams were anesthetized with pentobarbital before the embryos were removed and killed by immersion in Bouin’s fixative. After 24 h, the embryos were transferred to 10% neutral formalin until the time of embedding in either paraffin or methacrylate. The blocks were serially sectioned (every 10th was saved) at 6 μm (paraffin) or at 3 μm (methacrylate) in the coronal, sagittal, and horizontal planes. For \[^{3}H\]thymidine autoradiography, the slides were coated with Kodak NTB-3 emulsion, exposed for 6 weeks (paraffin sections) or 12-18 weeks (methacrylate sections), developed in Kodak D-19, and poststained with hematoxylin and eosin.

RESULTS

A Note on Terminology

In this paper and its companion (9), we use the term limbic cortex to include parts of Broca’s limbic lobe that have been traditionally considered neocortex by some (23, 31, 32) and variously called allocortex, peripaleocortex, periarchicortex, proisocortex, etc., by specialists in cytoarchitectonics (1, 2, 40). The major components of the lateral limbic cortex are the insular, perirhinal, and gustatory areas above and in the rhinal sulcus in the lateral wall. The major components of the medial limbic cortex are the dorsal peduncular, infralimbic, cingulate, and retrosplenial areas in the medial wall. The two join in the orbital areas below the frontal pole.

The insular cortex forms the dorsal border of the piriform cortex. Zilles (47) describes two areas anteriorly, ventral agranular insular (AIV) in the rhinal sulcus and dorsal agranular insular (AID) just above AIV in the lateral cortical wall. The insular cortex continues posteriorly in the rhinal sulcus as area AIP (posterior agranular insular). Both AIV and AIP are similar to Krieg’s (26, 27) area 13, while AID is a more circumscribed part of Krieg’s area 14. Krieg (27) describes three cortical layers throughout the insular cortex, an outer layer of small granular type cells, an intermediate layer of pyramidal cells, and a deep layer with horizontally flattened cells. All areas of the insular cortex overlie the claustrum which makes this area homologous to the human insular cortex (27). The gustatory cortex, named GU by Zilles (47), is defined by its reciprocal connections to the taste area in the medial part of the thalamic ventromedial nucleus (16, 21, 29, 35, 36, 41, 43, 46). GU forms the dorsal border of AID and is incorporated into Krieg’s (26, 27) area 14. The cellular layers are more distinct and a sparse but definite layer IV can be distinguished, similar to what is seen in the secondary somatosensory area (48). Posterior to the insular cortex, the perirhinal cortex (PR, Ref. (47); area 35, Refs. (26, 27)) lies in the rhinal sulcus just below the auditory cortex and above the entorhinal cortex. The cortical layers are thinner, and the horizontally oriented cells in layer VI are especially prominent.

Neurogenesis in Insular, Perirhinal, and Gustatory Areas

The Overall Ventral to Dorsal Neurogenetic Gradient

When \[^{3}H\]thymidine injections are given on E18 and E19, cell labeling patterns in the lateral cortical wall at level A8.2 (Fig. 1) indicate that only a thin band of superficial cells are labeled in the ventral agranular insular cortex in the rhinal sulcus (Fig. 1C), while the dorsally located gustatory cortex (Fig. 1B) has a considerably thicker band of superficial labeled cells. Besides indicating that deep cells are older than superficial cells (a neurogenetic gradient found throughout the entire cortex), these labeling patterns also indicate a strong ventral (older) to dorsal (younger) neurogenetic gradient. Since that neurogenetic gradient is found throughout the entire rostrocaudal expanse of the AIV, AID, and gustatory (GU) areas, only the data for level A8.2 (drawing, Fig. 2) are shown as an illustration. Within layers II–IV (right column of graphs, Fig. 2), neurogenesis occurs earlier in AIV than in GU (P < 0.0001) and earlier in AID than in GU (P < 0.0001). The gradient is quite powerful when AIV and GU are compared. For example 59% of the laminae II–IV cells originate on or before E16 in AIV, while only 26% of these cells originate during this time in GU. Within layer V (left column of graphs, Fig. 2), neurogenesis occurs earlier in AIV than in AID (P < 0.0003) and earlier in AID than in GU (P < 0.002). Again, there are sharp differences between AIV and GU. For example 75% of the layer V cells originate on or before E15 in AIV, while only 47% of these cells originate during the same time period in GU. Layer VI neurogenesis occurs mainly on E14 (16%) and E15 (65%) simultaneously in GU, AID, and AIV (all comparisons P > 0.05; data are not shown). In the deep parts of AIV and AID, fewer cells are generated on E14 and more on E15 than would be expected by the pattern seen in GU. Possibly some of the oldest deep cells are missing in AID and AIV. That
FIG. 1. Photomicrographs of the lateral cortical wall at level A8.2 in an animal exposed to [3H]thymidine on E18 and E19 and killed on P60. (A) Low magnification view showing the increase in the depth of labeled cells (cells that appear solid black) progressing from the ventral agranular insular cortex (AIV) through the dorsal agranular insular cortex (AID) and into the gustatory cortex (GU). The areas encircled with dashed lines are shown at higher magnifications in B and C (6-μm paraffin section, hematoxylin/eosin stain).

FIG. 2. Neurogenesis in layer V (left column of graphs) and layers IV-II (right column of graphs) in the ventrolateral cortical wall at level A8.2. All bar graphs are the proportion of cells that are generated in a single day of embryonic life (see Table 1). There is a ventral (older) to dorsal (younger) neurogenic gradient (arrow in drawing) between areas in both sets of laminae.
circumstance is most likely due to the presence of the claustrum, which lies beneath the deep cells in AIV and AID. Claustral cells originate mainly on E15 and E16 (Bayer, in preparation). Because the borders are clear, no claustral cells were counted as part of layer VI.

**Rostral to Caudal Neurogenetic Gradients**

*Cortex in the rhinal sulcus.* The cortex in the rhinal sulcus extends nearly the entire length of the cerebral hemispheres (drawings, Fig. 3). Beginning with the anterior end, there is the AIV (A9.2–A7.2), the AIP (A6.2), and finally the perirhinal (PR, A4.2–A1.2). The cortex at level A5.2 is transitional between AIP and PR. Posterior to level A1.2, the entorhinal cortex is found in the rhinal sulcus. All layers have the same pattern of neurogenesis along the rostrocaudal plane, and the data in Fig. 3 show only those of layer V as an example. Cells in layer V are generated in a “sandwich” gradient such that the older cells are found in posterior AIV and in AIP (middle graph, Fig. 3); these cells originate significantly earlier than those in anterior AIV (top graph, Fig. 3; $P < 0.002$) or in PR (bottom graph, Fig. 3; $P < 0.0001$). The sandwich trend in cell labeling is found in 20 of 21 rats in the E16+E17 and E17+E18 injection groups which accounts for the high levels of significance given by the sign test. However, the magnitude of the gradient is low with a 10–14% divergence in neurogenesis between areas: approximately 83% of the AIV/AIP cells originate on or before E15, while fewer cells originate during this same time period in AIV (73%) and PR (69%).

*Dorsal agranular insular cortex.* The dorsal agranular insular cortex (AID) extends from levels A9.2 to A7.2 (drawings, Fig. 4A). Only cells in layer VI have a significant caudal (older) to rostral (younger) neurogenetic gradient ($P < 0.0001$; two bottom graphs, Fig. 4A). Nearly 23% of the layer VI cells at levels A8.2–A7.2 originate on or before E14, while only about 11% of these cells originate during this same period at A9.2. There are no gradients along the rostrocaudal plan in layers V, IV, and II–III (all $P > 0.05$) and their data are combined in

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**FIG. 3.** The time of origin of layer V neurons in the rhinal sulcus between levels A9.2 (top drawing) and A1.2 (bottom drawing). Bar graphs represent the proportion of neurons originating on single days during embryonic life. The derivation of the data shown in the bottom graph is given in Table 1. The oldest neurons in A7.2–A6.2 are “sandwiched” by younger neurons anteriorly and posteriorly (large bifurcating arrow in drawings).
the three top graphs of Fig. 4A. There is a highly significant deep (older) to superficial (younger) neurogenetic gradient between layers (all comparisons, $P < 0.0001$). Layer VI has peak neurogenesis on E15, layer V on E15–E16, layer IV on E16–E17, and layers II–III on E16–E18.

**Gustatory cortex.** The GU extends from A9.2 through A6.2 (drawings, Fig. 4B). Both layers VI and V have neurogenetic gradients along the rostrocaudal plane. Cells in layer VI at levels A8.2–A5.2 (bottom graph, Fig. 4B) originate significantly earlier than those at level A9.2 (fifth graph from the top, Fig. 4B; $P < 0.0001$). These differences are best seen in the amount of neurogenesis that occurs on or before E14 (A8.2–A5.2, 24%; A9.2, 13%). Cells in layer V at levels A7.2–A5.2 (fourth graph from the top, Fig. 4B) originate significantly earlier ($P < 0.002$) than those at levels A9.2–A8.2 (third graph from the top, Fig. 4B). Neurogenetic differences in layer V are best seen in the amount of cells that are generated on or after E16 (A7.2–A5.2, 36%; A9.2–A8.2, 53%). Since layers IV and II–III originate simultaneously at all levels (all $P > 0.05$), their data are combined (two top graphs, Fig. 4B). There is a highly significant deep (older) to superficial (younger) neurogenetic gradient between layers (all comparisons, $P < 0.0001$). Layer VI has peak neurogenesis on E15, layer V on E15–E16, layer IV on E16–E17, and layers II–III on E17–E18.

**Similarities between Neurogenetic Patterns in Medial and Lateral Limbic Cortices**

A companion paper presented data indicating that the medial limbic cortex also contains a prominent ventral (older) to dorsal (younger) neurogenetic gradient in the medial wall (Fig. 6A in Ref. (9)). The most ventral part
of the medial limbic cortex, the dorsal peduncular cortex, is especially early in time of origin. To see if there is an anterior continuation between the "oldest" medial and lateral parts of the limbic cortex, a series of sagitally sectioned brains were used to quantify the times for neurogenesis in the cortex tucked under the frontal pole and lying dorsal to the olfactory peduncle. Krettek and Price (25) called this area the orbital cortex, a name also used by Zilles (47). In sections 1.5 mm lateral to the midline (33), the orbital cortex sharply curves into the olfactory peduncle without a break. Just as for the medial and lateral limbic cortical areas, there is a tendency for cell layers to become thinner and less definite with increasing distance from the somatic neocortex. Counts were made in laminae VI, V, and IV–II in two strips of orbital cortex, one just dorsal to the sharp curve into the olfactory peduncle and the other just beneath the frontal pole. The data combined for all laminae in each strip are shown in Table 2 and indicate that the cortex located nearer to the olfactory peduncle is generated earlier (34% have accumulated on or before the morning of El6) than the cortex located nearer to the frontal pole (25% have accumulated on or before the morning of El6). The neocortex in the frontal pole is generated later (13). Thus, the orbital cortex appears to be the anterior contact zone between the medial and lateral limbic "rim" around the neocortex.

### TABLE 2

<table>
<thead>
<tr>
<th>Day of origin</th>
<th>Ventral orbital % cells originating</th>
<th>Dorsal orbital % cells originating</th>
</tr>
</thead>
<tbody>
<tr>
<td>E13</td>
<td>1.38</td>
<td>0.31</td>
</tr>
<tr>
<td>E14</td>
<td>4.31</td>
<td>4.86</td>
</tr>
<tr>
<td>E15</td>
<td>28.54</td>
<td>19.43</td>
</tr>
<tr>
<td>E16</td>
<td>34.67 (34.23%)</td>
<td>30.47 (24.59%)</td>
</tr>
<tr>
<td>E17</td>
<td>17.40</td>
<td>29.41</td>
</tr>
<tr>
<td>E18</td>
<td>9.65</td>
<td>8.80</td>
</tr>
<tr>
<td>E19</td>
<td>3.39</td>
<td>7.31</td>
</tr>
<tr>
<td>E20</td>
<td>0.65</td>
<td>0.42</td>
</tr>
</tbody>
</table>

*Cells generated during each embryonic day in strips (combined layers II–VI) of the orbital cortex beneath the frontal pole. Cell counts were done in sagittally sectioned brains of animals exposed to [3H]thymidine on 2 consecutive days of embryonic development and killed on P90. These data were calculated as described in Table 1. The numbers in parentheses are the proportions of neurons that have already originated by the morning of El6. The ventral orbital cortex is nearer to the olfactory peduncle and accumulates neurons earlier than either the dorsal orbital cortex (P < 0.002) or the frontal pole (P < 0.038). The "Young Center" Sandwich Gradient between Limbic Cortex and Neocortex

The level just anterior to the genu of the corpus callosum (Fig. 5) was chosen for analysis since that area contains the most extensive representation of the medial limbic cortex (ML), a central expanse of somatic neocortex (NC), and a wedge of lateral limbic cortex (LL) just above the rhinal sulcus. To give an overview of neurogenetic patterns within and between the limbic cortex and the neocortex, embryos were killed on E22 after administering single [3H]thymidine injections during various days of cortical neurogenesis (Fig. 5). In embryos injected on El7 (Fig. 5A), only thin bands of superficial cells are labeled both laterally and medially in the limbic cortices, while the neocortex has a thicker band of labeled cells. An El8 injection (Fig. 5B) labels only a few cells in the most ventral medial limbic cortex (high magnification view in Fig. 6A) and the lateral limbic cortex in the rhinal sulcus (Fig. 6C), more cells in the centrally located neocortex (Fig. 6B). An El9 injection (Fig. 5C) labels cells scattered throughout the cortex, again more numerous in the neocortex than in either the medial or lateral limbic cortices. These labeling patterns indicate that neurogenesis is completed earlier in the ventral extremes of the limbic cortex than in the neocortex.

Subplate and Cortical Plate Morphogenesis in the Limbic Cortex

From E18 on into the early postnatal period, the subplate forms a separate layer just beneath the cortical plate (12). It is noteworthy that the medial and lateral extremes of the cortical plate, presumably the areas which will differentiate into the medial and lateral limbic cortices, do not have an underlying subplate. Since substantial subplate neurogenesis occurs on E15 (12), a single injection of [3H]thymidine on that day will heavily label many subplate neurons. A low magnification view of an E21 embryo exposed to [3H]thymidine on E15 (Fig. 7D) shows that the subplate (SP) stands out as a band of intensely labeled cells. Higher magnification views show that the subplate is especially prominent in central areas.
which will differentiate into neocortex (Fig. 7B). Both medially (Fig. 7C) and laterally (Fig. 7A), the intensely labeled cells (arrows) are incorporated into the depths of the cortical plate rather than in a separate subplate (shown in the drawing in Fig. 7E).

DISCUSSION

Subplate and Cortical Plate “Blending” in the Limbic Cortices

A study in cats (30) and our work in rats (12) presented observations consistent with the hypothesis that the first cortical plate cells are actually destined for the subplate. On E18 and E19 in rats, subplate cells “delaminate” from the cortical plate and form a separate layer (12). Curiously, that does not occur in regions of the cortical plate that will presumably develop into the limbic cortical areas (Figs. 7D and 7E); rather, the deep layers in lateral and medial limbic cortical areas have unusually older cells (arrows, Figs. 7A and 7C) that are generated concurrently with those in the subplate (12). Another corollary is that subplate cells are oriented horizontally.

1 Nearly all subplate neurogenesis occurs on E14 and E15 (12). In posterior parts of both the dorsal agranular insular and gustatory cortices, nearly 20% of the cells in layer VI are generated on E14 (23–24%; bottom graphs, Figs. 4A and 4B) and nearly 40% in the posterior agranular insular cortex (data are not illustrated); throughout the ventral lateral limbic cortex, neurogenesis is essentially complete on E15. Where a subplate is separate in the somatic neocortex, few layer VI cells are generated on E14 (<10%), more on E16 (25–55%) and E17 (between 30 and 50% in medial areas (13)). Although the neurogenetic gradients in layer VI of both the lateral limbic and neocortices are sequential, there is a “supernormal” accumulation of older deep cells in the lateral limbic cortex. Similarly, it is only in the medial limbic cortex that between 30 and 50% of the layer VI cells are born on or before E15 (9, 13). These early cells may be the “infiltrated” subplate neurons.
FIG. 8. The rat cerebral cortex realistically drawn from the lateral and medial aspects (small drawings in upper right and upper left), and a diagrammatic view of the entire cortical mantle where the top and all sides are represented in one plane (large middle drawing). The neocortex is completely surrounded by two overlapping belts. The outer belt (in reality, most ventral) is continuous anteriorly with the olfactory peduncle and contains paleocortex below the rhinal sulcus and in the posterior wall (anterior, APO, and posterior, PPO primary olfactory cortices; lateral, ECL and medial, ECM entorhinal cortices). The paleocortex continues without a break into the archicortex (hippocampus, HP) that extends beneath the corpus callosum in the medial wall. An inner belt (in reality, more dorsal) is open posteriorly and contains the lateral limbic cortex in and above the rhinal sulcus (perirhinal, PR; insular, AIP, AIV, AID; gustatory, GU), the medial limbic cortex in front of and above the corpus callosum (dorsal peduncular, DP, infralimbic, IL, cingulate, CG, retrosplenial, RS). Both limbic cortices join in the orbital cortex (OC) below the frontal pole. Directions in neurogenetic gradients are represented by arrows pointing from areas containing older cells to areas containing younger cells and are drawn according to data presented here for the lateral limbic cortex and elsewhere for the other cortical areas. Each major cortical subdivision has characteristic arrows (see legend). Note that foci of "old" cells are found in the limbic cortical belt and in the paleoarchicortical belt but not in the neocortex. In addition, the ventral parts of the limbic cortical belt in the anterior third of the hemisphere are older than the nearest sector of neocortex (solid black arrows).

rather than radially, and the deep layers of both the medial (9) and lateral limbic cortices have a predominance of cells with horizontal orientations.

Subplate/cortical plate blending in limbic areas may also be related to the distribution of dopamine axons in the cortex, especially those from the ventral tegmental area. Dopaminergic axons terminate predominantly in limbic rather than neocortical areas (14, 15, 18, 19, 21, 24, 34, 42, 44). The small dopamine projection to the neocortex comes predominantly from the ventral tegmental area and terminates only in the very deepest layers (VIb or VII) of the neocortex (18), just where remnants of the subplate are found in adult brains. The ventral tegmental area projection spreads out in the deep layers (V–VI) of the limbic cortical areas (18, 20), just what would be expected if subplate cells infiltrated the deep layers in the limbic cortices.

Reversal of Neocortical Gradients in the Medial Limbic Cortex

One of the most prominent neurogenetic gradients found in the neocortex is that older cells are in ventrolateral areas and younger cells are in dorsomedial areas (13). If the medial limbic cortex is a direct continuation of the neocortex, one would expect to see the youngest cortical areas in the most ventral parts of the medial limbic cortex. It is striking that exactly the opposite is found. Throughout the medial limbic cortex, superficial cells have a ventral (older) to dorsal (younger) gradient (9, 37, 38). Subplate incorporation into the medial limbic
cause of divergence in ontogenetic patterns, while the limbic cortex is shown by their sharing sandwich neurogenetic gradients: older areas are bordered anteriorly and posteriorly by younger areas. In contrast, the neocortical system diverges by showing "open" neurogenetic gradients, where older areas are either lateral or anterior and younger areas are either medial or posterior (13). The limbic cortex has two foci of older cells, one laterally (AIP; center graph, Fig. 3), another medially (anterior CG, DP, and IL in Fig. 8) (9). Younger cells are in areas anterior and posterior to these foci (arrows, Fig. 8). In the paleo- and archicortical systems, there is a single focus of older cells in the posterior primary olfactory cortex (PPO, 8). Cells are born later in the anterior primary olfactory cortex the closer they are to the olfactory bulb (8). Similarly, cells are born later as their positions shift away from PPO through lateral and medial entorhinal areas into the hippocampus (6).

It is noteworthy that the sandwich gradients in systems 1 and 2 "line up" according to age in the ventral/dorsal plane. That is best seen in the lateral cortical wall. The focus of oldest cells in the PPO is just beneath the AIP. In AIV, AIP, and GU, deep cells have a posterior (older) to anterior (younger) neurogenetic gradient (Figs. 3 and 4) matching a similar gradient in the segment of paleocortex beneath them. The youngest parts of the anterior primary olfactory cortex are closest to the anterior focus of young cells in the limbic cortex just above the olfactory peduncle (ventral and dorsal orbital areas, Table 2). Behind AIP and PPO, younger cells in the PR are above younger cells in the lateral entorhinal cortex.

An ontogenetic link between the neocortex and the limbic cortex is shown by their sharing neurogenetic gradients: older areas are bordered anteriorly and posteriorly by younger areas. In contrast, the neocortical system diverges by showing "open" neurogenetic gradients, where older areas are either lateral or anterior and younger areas are either medial or posterior (13). The limbic cortex has two foci of older cells, one laterally (AIP; center graph, Fig. 3), another medially (anterior CG, DP, and IL in Fig. 8) (9). Younger cells are in areas anterior and posterior to these foci (arrows, Fig. 8). In the paleo- and archicortical systems, there is a single focus of older cells in the posterior primary olfactory cortex (PPO, 8). Cells are born later in the anterior primary olfactory cortex the closer they are to the olfactory bulb (8). Similarly, cells are born later as their positions shift away from PPO through lateral and medial entorhinal areas into the hippocampus (6).

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An ontogenetic link between the neocortex and the limbic cortex is shown by their sharing neurogenetic gradients in the radial direction (not shown in Fig. 8). Without exception cells in deep laminae originate earlier than cells in superficial laminae. That is the "inside-out" gradient first seen by Angevine and Sidman (5) and confirmed in every subsequent thymidine autoradiographic study of neocortical development. In contrast, the paleocortex and parts of the archicortex have exceptions to that gradient. The split between the limbic cortex...
and the paleo- and archicortices on the "inside-out" gradient may be related to the embryonic cortical plate. The ventrolateral extreme of the cortical plate (Figs. 5 and 6) differentiates into the lateral limbic cortex. The piriform cortex appears to differentiate from the lateral ganglionic eminence in the basal telencephalon rather than from the cortical plate (Altman and Bayer, in preparation). The cortical plate on the medial wall is highly modified in the region of the developing hippocampus, and the dentate gyrus does not develop from it (7).

A summary of the ontogenetic features in cortical systems (Fig. 9) suggests a simpler phylogenetic scheme where the link between systems is centered in the limbic cortex. Directions in neurogenetic gradients indicate that the paleo- and archicortices (System 1) and the neocortex (System 3) are nearly completely divergent. We postulate that these two ontogenetic systems have unique phylogenetic sources. In support of Herrick's (22) suggestion, one phylogenetic source probably gives rise to the neocortex, since it is the only cortex with "open" neurogenetic gradients. Although they are traditionally considered to evolve from separate primordia (1, 2, 22, 40), the striking sequential nature of neurogenesis throughout the combined paleocortex and archicortex (System 1) is the basis for our suggestion that it is a single ontogenetic field and may have a single phylogenetic source. Finally, there is strong ontogenetic evidence for a dual phylogenetic origin to the limbic cortex. We suggest that one source is in the combined paleo- and archicortices and the other is in the neocortex. It would be intriguing to test the hypothesis further by doing autoradiographic analyses in a "primitive" mammalian brain, such as the cat, and in a more "advanced" brain, such as the rat, to see whether the ontogenetic systems in the rat cerebral cortex are found more generally in the cerebral cortices of other mammals.

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